

Specific requirement for ATP at an early step of *in vitro* transcription of human mitochondrial DNA

(imidonucleotides/promoters/heavy strand/light strand/RNA polymerase)

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ABSTRACT The ATP concentrations allowing transcription of both heavy- and light-strand of human mtDNA in a HeLa cell mitochondrial lysate were found to cover a broad range, with a maximum around 2.5 mM, and with reproducible differences in the ATP response curves for the two transcription events. Direct measurements showed that nonspecific ATP degradation during the assay did not account for the high ATP requirement. 5'-Adenylyl imidodiphosphate (p[NH]ppA), an ATP analog with a nonhydrolyzable β - γ bond, was unable to substitute for ATP in supporting mtDNA transcription but greatly stimulated this transcription in the presence of a low concentration of exogenous ATP. Evidence was obtained indicating that p[NH]ppA did not support an early event in mtDNA transcription (formation of preinitiation complex or initiation), whereas this analog could substitute effectively for ATP in the subsequent elongation steps. These results pointed to a specific requirement for ATP at an early step of the transcription process.

Recently, soluble transcription systems have been developed from mitochondria of yeast (1), mammalian cells (2–4), and *Xenopus laevis* oocytes (5), which are capable of transcribing the homologous mitochondrial DNA (mtDNA) with absolute specificity. In particular, a mitochondrial RNA polymerase activity capable of initiating transcription at the heavy (H)-strand rRNA promoter and at the light (L)-strand promoter of human mtDNA has been identified and partially purified from HeLa cells (3, 4) and KB cells (2, 6).

A characteristic feature of the human mitochondrial RNA polymerase is that the ATP concentration required for maximum activity (0.5–1.0 mM) is 15- to 20-fold higher than that of the other NTPs (4). A similar optimum ATP concentration has been found in a transcription system using isolated HeLa cell mitochondria (7, 8). Furthermore, the ATP concentration in HeLa cell mitochondria *in vivo* has been estimated to be close to the optimum for mtDNA transcription in the two systems mentioned above (9). However, the specific biochemical step(s) in the transcription process that requires a high ATP concentration has not been identified. In the present work, the nature of the ATP requirements for mtDNA transcription has been investigated in a HeLa cell mitochondrial lysate. The existence of a specific requirement for ATP, which cannot be satisfied by 5'-adenylyl imidodiphosphate (p[NH]ppA), at an early step of mtDNA transcription has been demonstrated.

MATERIALS AND METHODS

Preparation of Mitochondrial Lysates. Mitochondrial lysates were prepared essentially as described (4), except that the final mitochondrial pellet was resuspended in one-half the

volume of the total whole cell pellet of a medium containing 25 mM Hepes (pH 7.6), 5 mM MgCl₂, 0.5 mM EDTA, 1 mM dithiothreitol, 10% (vol/vol) glycerol, and 1 mM phenylmethylsulfonyl fluoride.

mtDNA Template. The template used in the *in vitro* transcription assays was a 1022-base-pair fragment, containing the H-strand and L-strand transcription initiation sites, derived from the plasmid pmt-H8 described previously (4) by digestion with *Nar* I and *Hind* III (see Fig. 1).

In Vitro Transcription Assays. The standard transcription reaction mixture consisted (except where noted) of 10 mM Tris-HCl pH 8.0 (30°C)/10 mM MgCl₂/1 mM dithiothreitol/10% glycerol/bovine serum albumin (100 μ g/ml)/0.5 mM ATP/0.1 mM GTP/0.1 mM CTP/0.01 mM unlabeled UTP/10 μ Ci of [α -³²P]UTP (410 Ci/mmol; 1 Ci = 37 GBq)/10–40 μ g of template per ml, in a final vol of 100 μ l. The mitochondrial lysate was added to 10% of the reaction volume, unless otherwise specified, and the mixtures were incubated at 30°C for 30 min. The transcription products were extracted as described (4) and electrophoresed on 5% polyacrylamide/7 M urea gels unless stated otherwise.

ATP Degradation Assays. The extent of ATP degradation during *in vitro* transcription was measured by ion-exchange thin-layer chromatography of the reaction mixtures at various time points during the reaction. Chromatography was carried out essentially as described by Randerath and Randerath (10), using poly(ethyleneimine)-cellulose (PEI-cellulose) thin-layer plates (Brinkmann).

RESULTS

Description of the System. Fig. 1 shows a schematic map of the linear mtDNA template used in the present work to generate H-strand and L-strand transcripts *in vitro*. In transcription assays using this template, an \approx 550-nucleotide (nt) runoff transcript originating at the H-strand rRNA promoter and an \approx 318-nt runoff transcript originating at the L-strand promoter (3, 6) were reproducibly obtained (Fig. 2). The labeled band migrating at \approx 950 nt and the minor slower moving bands represent labeled RNA species (the 950-nt band being presumably 12S rRNA), as shown by the “–DNA” controls (Fig. 3) and by RNase and DNase digestion tests. The nature of this RNA labeling activity, which is independent of the ATP concentration in the incubation mixture, has not been investigated further.

Effects of Lysate and Template Concentration on the Pattern of H-Strand and L-Strand *in Vitro* Transcription. For all template concentrations tested, the maximum labeling of the L-strand transcript occurred at a lysate concentration of 1/10th to 1/20th of the reaction volume, with a marked decrease at a higher (1/3rd) or lower (1/40th) lysate concentration (Fig. 2). In contrast, relatively small changes in the labeling of the H-strand transcript were observed for lysate

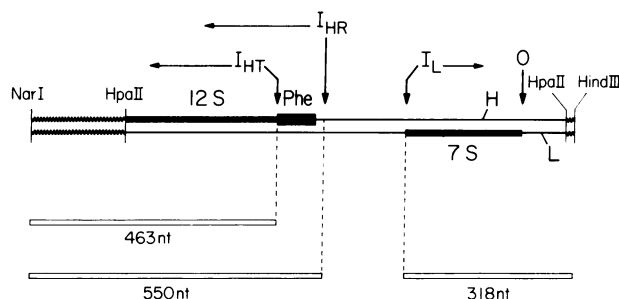


FIG. 1. Schematic representation of the human mtDNA fragment used as a template to generate runoff transcripts with a HeLa cell mitochondrial lysate. The *Hpa* II-8 fragment of human mtDNA was excised from the plasmid pmt-H8 (4) as a *Nar* I/*Hind* III fragment. Downward arrows indicate the H-strand transcription initiation sites (I_{HR} and I_{HT}), the L-strand transcription initiation site (I_L), and the origin of H-strand synthesis (O) (11-13). Leftward and rightward arrows show the direction of H-strand and L-strand transcription. Wavy lines represent portions of the pUC9 vector from pmt-H8 remaining in the excised fragment. The positions and expected sizes of the runoff transcripts are indicated in the lower part of the figure.

concentrations varying between 1/3rd and 1/10th, with a decrease at the lower lysate concentrations (1/20th to 1/40th). (The labeling of the runoff transcripts and endogenous RNA bands in the seventh lane is probably artificially high.) Therefore, the peak of transcriptive activity occurred at a lower lysate concentration for L strand than for H strand. Furthermore, for each lysate concentration, H-strand transcription tended to reach a maximum at a lower DNA concentration than L-strand transcription, as shown for the 1/10th to 1/40th lysate concentrations.

Effects of ATP Concentration on H-Strand and L-Strand *in Vitro* Transcription. Fig. 3 shows the electrophoretic patterns of the transcripts synthesized during a 30-min incubation in assays carried out as described using 20 μ g of template per ml. As previously found for mtDNA transcription by partially purified mitochondrial RNA polymerase (4), RNA synthesis in a crude mitochondrial lysate is completely dependent on added ATP. From the electrophoretic patterns and from a densitometric analysis of appropriate exposures of the autoradiograms (Fig. 4a), one can see that there is a progressive increase in the efficiency of both H-strand and L-strand transcription in the presence of increasing ATP concentrations, with a maximum around 2.5 mM and a shoulder around 0.5-1.0 mM. However, the shapes of the ATP response

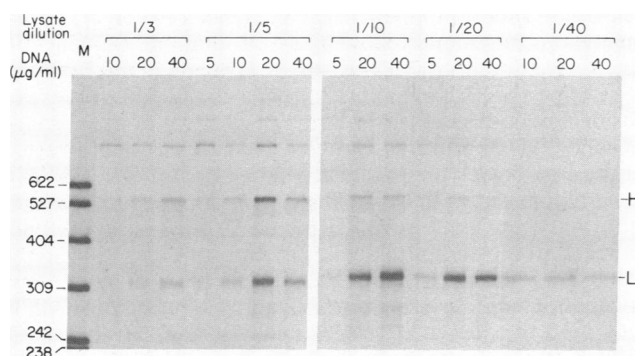


FIG. 2. Effects of lysate and template concentration on *in vitro* transcription of human mtDNA by a HeLa cell mitochondrial lysate. *In vitro* runoff transcripts were synthesized as described, except that the ATP concentration was 1.5 mM and the template and lysate concentrations in the final incubation mixture varied as indicated. H, H-strand runoff transcript; L, L-strand transcript; M, end-labeled *Msp* I digest of pBR322 DNA. (Sizes of fragments are given in base pairs.)

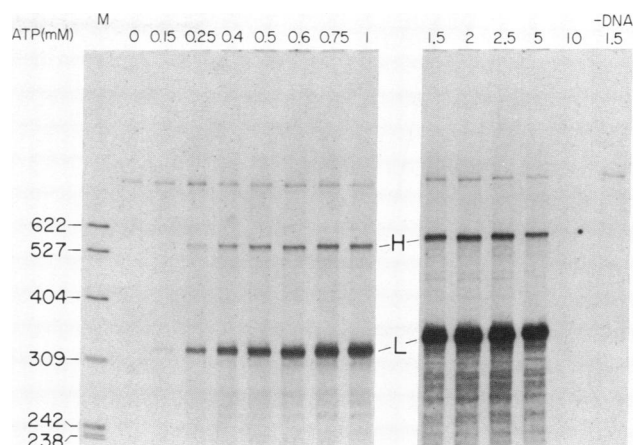


FIG. 3. Effects of ATP concentration on H-strand and L-strand transcription efficiency. *In vitro* transcription reactions were carried out as described using 20 μ g of template per ml in the presence of various concentrations of ATP for 30 min at 30°C. Symbols are the same as in Fig. 2.

curves for the H-strand and L-strand transcription events are reproducibly different.

It had been previously observed that the partially purified RNA polymerase has maximal activity at 0.5-1.0 mM ATP and half-maximal activity at $\approx 85 \mu$ M ATP (4). It seemed possible that the shift to higher ATP concentrations for optimum efficiency of transcription observed in the crude mitochondrial lysate was due to extensive degradation of the exogenous ATP by the ATPase(s) or phosphatase(s) present in the crude lysate. Direct measurements of the quantitative behavior of [α - 32 P]ATP in the lysate, under the conditions used in the transcription assays (in the presence of 0.25 mM ATP), showed indeed a progressive degradation of the nucleotide, which reached $\approx 20\%$ of the original level after 30 min (Fig. 5). In the presence of 1.0 mM and 2.5 mM ATP, [α - 32 P]ATP degradation was somewhat faster than in 0.25 mM ATP during the first 5-min incubation, but it became slower over the next 25 min, so that $\approx 40\%$ or $\approx 65\%$ of the original level of [α - 32 P]ATP was still intact after 30 min in the presence of 1.0 mM or 2.5 mM ATP, respectively (Fig. 5).

The above observations tended to argue against the possibility that ATP degradation was responsible for the high

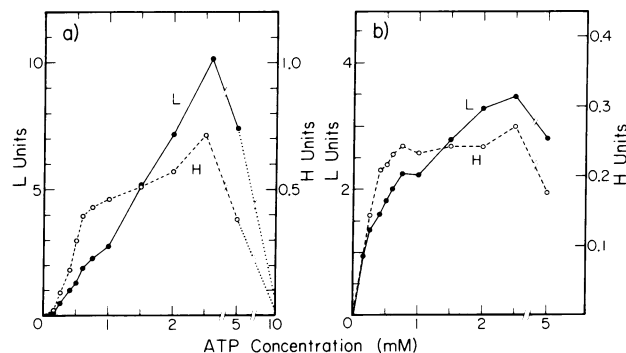


FIG. 4. Quantitation of H- and L-strand transcription efficiency in the presence of various concentrations of ATP. (a) Autoradiograms shown in Fig. 3 were scanned with an LKB Ultrosan XL densitometer. The area (in arbitrary units) under the peaks for H-strand (H units) and L-strand (L units) transcripts is plotted against ATP concentration (mM). (b) Transcription reactions were carried out as described in the legend for Fig. 3, except that the reaction was for 5 min at 30°C. Autoradiogram (not shown) was scanned and transcription was quantitated as described in a.

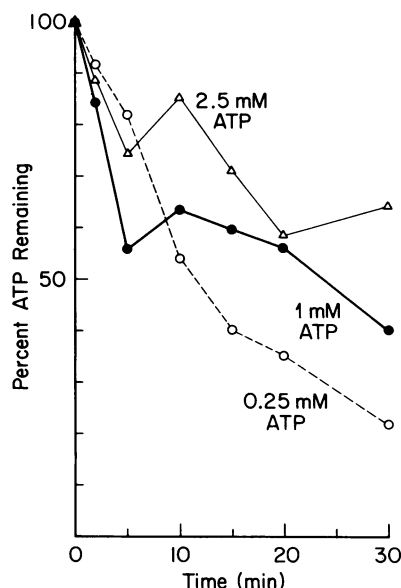


FIG. 5. Course of ATP degradation during *in vitro* transcription reactions carried out on a mtDNA template by a HeLa cell mitochondrial lysate. Transcription reactions were performed in 100- μ l reaction volumes containing 10 μ l of the mitochondrial lysate; 20 μ g of template per ml; GTP, CTP, and UTP (0.1 mM each); [α - 32 P]ATP (10 μ Ci per reaction mixture); and 0.25 mM, 1 mM, or 2.5 mM ATP. At various times, 10 μ l of the reaction mixture was removed, brought to 0.2% NaDodSO₄, and immediately frozen at -80° C. Two microliters of each reaction mixture was spotted on a PEI-plate and chromatographed as described. After autoradiography, the spot corresponding to ATP was cut out and counted.

ATP requirement for maximum transcription efficiency in a crude lysate. To verify this interpretation, the ATP effect was measured in 5-min transcription assays, during which, because of the linearity of the transcription reaction (4), the average ATP level was expected to be only 10–20% lower than the original. The results are shown in Fig. 4b. Although the ATP response curves obtained for H-strand and L-strand transcription showed a broadening toward the 0.5–1.0 mM shoulder, their general shape and their characteristic differences were conserved. In other experiments, it was observed that, in contrast to the effects of ATP concentration, varying the concentration of GTP between 0.1 mM and 2 mM had no influence on the efficiency of transcription.

p[NH]ppA Cannot Substitute for ATP in Supporting *in Vitro* Transcription of mtDNA. It has been shown that in a whole HeLa cell extract (14) or in a reconstituted HeLa cell system (15), ATP plays two essential roles for RNA polymerase II activity—namely, as a substrate for the polymerase and as a compound with an energy-rich phosphate bond. In particular, it has been shown that hydrolysis of the β - γ bond of ATP is required for an early event in the transcription process (14, 15). To test for such a requirement in the *in vitro* mtDNA transcription system, the effect of replacing ATP by p[NH]ppA, an ATP analog with a nonhydrolyzable β - γ bond (16), was investigated. As shown in Fig. 6a, the addition of increasing concentrations of p[NH]ppA (up to 1.5 mM) to the incubation mixture instead of ATP did not promote any detectable level of H-strand and L-strand transcription. By contrast, in the presence of a relatively low concentration of ATP (0.15 mM), p[NH]ppA was able to greatly stimulate the incorporation of [32 P]UMP into H-strand- and L-strand-specific transcripts, up to levels much higher than supported by 0.15 mM ATP alone. These levels, however, were only 15–20% of the levels reached with an equivalent concentration of ATP. The stimulation of transcription by p[NH]ppA

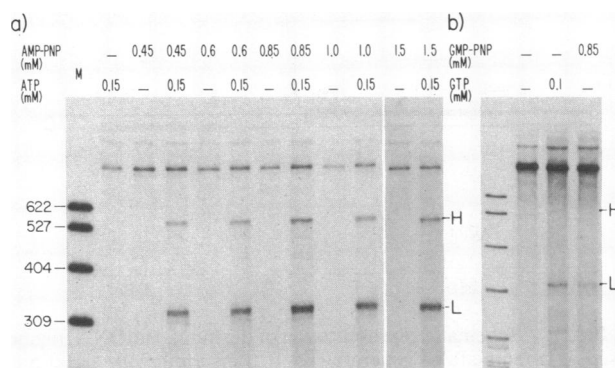


FIG. 6. Effects of nucleotide analogs on *in vitro* synthesis of runoff mtDNA transcripts by a HeLa cell mitochondrial lysate. (a) Effects of p[NH]ppA (AMP-PNP) (Boehringer Mannheim) in the absence or presence of 0.15 mM ATP. *In vitro* transcription reactions were carried out as described. (b) Effect of substituting p[NH]ppG (GMP-PNP) (Boehringer Mannheim) for GTP in the transcription reaction. Runoff transcripts were synthesized in a 100- μ l reaction volume containing 40 μ g of template per ml, 10 μ l of lysate, 0.25 mM ATP, 0.1 mM CTP, 0.01 mM UTP, 30 μ Ci of [α - 32 P]UTP per assay (410 Ci/mmol), and either no GTP (first lane), 0.1 mM GTP (second lane), or 0.85 mM p[NH]ppG (third lane). Autoradiography was carried out at -80° C with an intensifying screen for 44 hr (a) or 21 hr (b). Symbols are the same as in Fig. 2.

was concentration dependent, with the maximum being observed at \approx 0.85 mM p[NH]ppA.

The inability of p[NH]ppA to fully replace ATP in supporting *in vitro* transcription of mtDNA was not a general property of imidonucleotide analogs. In fact, as shown in Fig. 6b, another imidonucleotide, p[NH]ppG (16), could apparently replace GTP, although it was incorporated into L-strand transcripts with a lower efficiency (\approx 80%) than GTP.

p[NH]ppA Cannot Support an Early Event in the *in Vitro* mtDNA Transcription Reaction. A plausible interpretation of the observed effects of p[NH]ppA on mtDNA transcription *in vitro* was that this analog failed to support initiation of transcription (defined as formation of the first phosphodiester bond) or formation of the preinitiation complex, but it was able to support elongation of already initiated chains, although at a reduced rate. To establish this point more firmly, two-step reactions were carried out in which the requirements for the synthesis of short transcripts accumulating in the absence of GTP could be discriminated from the requirements for the subsequent elongation of these transcripts to full-length runoff products in the presence of GTP. The first guanylic acid residue following a U-containing sequence in the L-strand transcript is the 14th (\pm 1) nt and in the H-strand transcript, the 23rd (\pm 1) nt (17). It was found that because of residual endogenous GTP, during incubation in the absence of added GTP, besides the expected short transcripts arrested at the first detectable G (and presumably at the earlier Gs), several longer transcripts arrested at various subsequent G positions were synthesized. However, transcription did not proceed to the end of template. In fact, as shown in Fig. 7a (lane 2), when the reaction was terminated after a 15-min incubation in the absence of GTP and the presence of ATP, no runoff transcripts were seen (see also Fig. 6b, first lane). When, after the incubation in the absence of GTP and in the presence of ATP, the reaction was allowed to continue for 2 min in the presence of GTP, a clear band corresponding to the L-strand runoff product (arrow), but no H-strand runoff, was observed (Fig. 7a, lane 3). If the first incubation was carried out in the absence of template and GTP and in the presence of ATP, and the second incubation (2 min) took place in a complete mixture, no L-strand runoff was made in the second step (lane 4). Similarly, if the first step was carried out in the

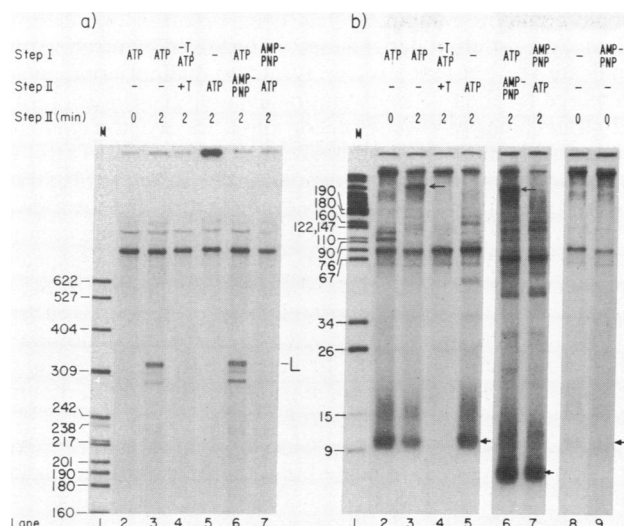


FIG. 7. Effects of ATP and p[NH]ppA (AMP-PNP) on early events in *in vitro* transcription. *In vitro* transcription reactions were performed in two steps. In step I, the template (40 μ g/ml) was incubated for 15 min at 30°C with 10 μ l of the mitochondrial lysate in a reaction vol of 100 μ l containing CTP (0.1 mM), UTP (0.01 mM), [α - 32 P]UTP (30 μ Ci per assay; 410 Ci/mmol), with the addition of 0.25 mM ATP or 0.85 mM p[NH]ppA, or omission of the template (–T), where indicated. After step I, the reaction was either terminated (a, lane 2; b, lanes 2, 8, and 9) or GTP (0.1 mM) alone (–), or together with template (+T) or ATP (0.25 mM) or p[NH]ppA (0.85 mM), as indicated, was added to allow elongation of transcripts to proceed for 2 min. Electrophoresis of the transcription products was carried out on a 5% polyacrylamide/7 M urea gel (a) or on a 15% polyacrylamide/7 M urea gel (b). Samples 6 and 7 in b were run on a separate gel, and samples 8 and 9 derive from a separate experiment. Symbols are the same as in Fig. 2. The pronounced band corresponding to a size of \approx 70 nt presumably represents end-labeled tRNAs. Note that, because of end-labeling, the pBR322 *Msp* I fragment strands are 2 nt longer than indicated in the figure.

absence of GTP and ATP and in the presence of template (lane 5), no runoff transcripts were observed, even after a 2-min second incubation in the presence of both nucleotides.

The experiments described above clearly indicated that the formation of the L-strand runoff transcripts depended on events occurring in both the first and the second step. This situation offered the opportunity to test whether p[NH]ppA could substitute for ATP in either of the two steps. As shown in Fig. 7a (lane 6), if the first incubation was carried out in the absence of GTP and in the presence of ATP, and the second incubation (2 min) was carried out in the presence of GTP and p[NH]ppA, a clear band corresponding to the L-strand runoff transcripts was visible, which was of similar intensity to that observed in the absence of p[NH]ppA in the second step (lane 3). On the contrary, when the template was incubated first in the absence of GTP and in the presence of p[NH]ppA, and then in the presence of GTP and ATP, no L-strand runoffs were formed (lane 7). The results of these experiments clearly showed that p[NH]ppA could not substitute for ATP in the first step of the two-step transcription assays.

Further insight into the biochemical events occurring in the two-step transcription assays described above was provided by an analysis of the incomplete transcription products by short electrophoretic runs (Fig. 7b). When the products of a 15-min reaction carried out in the absence of GTP and in the presence of ATP were thus analyzed, one observed the 14-nt-long L-strand transcript expected from termination at the first G following a U-containing sequence (Fig. 7b, lane 2, short arrow), but not the analogous 23-nt-long H-strand transcript. In addition, one could see several bands in the 100- to 200-nt size range, which presumably represented L-strand

transcripts terminated at various G positions; among these, two particularly strong bands corresponded to 110- to 120-nt-long transcripts. When, after the incubation in the absence of GTP and in the presence of ATP, the reaction was allowed to continue for 2 min in the presence of GTP, the 14-nt transcript band decreased markedly in intensity, while the 110- to 120-nt transcript bands disappeared, and the more slowly moving bands became more pronounced, simultaneous with the appearance of the runoff transcript (long arrow) (lane 3). When the first incubation was carried out in the absence of template and GTP and in the presence of ATP, and the second incubation was carried out in a complete mixture, no 14-nt transcript was synthesized in either the first or the second step (lane 4), indicating that the system was totally dependent on exogenous template. By contrast, when the first incubation took place in the presence of template but in the absence of GTP and ATP, the 14-nt L-strand transcript was still synthesized, presumably at the expense of the endogenous ATP pool (lane 8). (The samples analyzed in lanes 8 and 9 of Fig. 7b are derived from a separate experiment; these samples, like other samples from that experiment, corresponding to those run in lanes 2, 3, and 5–7, exhibited, for unknown reasons, a lower amount of the 14-nt product.) If, after a first step in the absence of GTP and ATP, the reaction was allowed to continue for 2 min in the presence of exogenous ATP, there was the appearance of slower moving bands in the 50- to 200-nt size range, which presumably corresponded to arrested L-strand transcripts; however, as mentioned above, there were no full-length transcripts (Fig. 7b, lane 5). The result of this experiment, when compared with that of the experiment in which ATP was already present in the first step (lane 3), strongly suggested that the runoff L-strand transcripts were formed, during the second incubation, from elongation of arrested transcripts that were longer than the 14-nt product.

When the first incubation was carried out in the absence of GTP and in the presence of ATP, and the second incubation was carried out in the presence of p[NH]ppA, the pattern of the short electrophoretic run (Fig. 7b, lane 6) was essentially identical to that observed when p[NH]ppA was absent in the second step (lane 3). When the template was incubated for 15 min in the absence of GTP and in the presence of p[NH]ppA, and the reaction then stopped, only the 14-nt transcript band was observed (lane 9), which was of the same intensity as in the analogous experiment in the absence of ATP and p[NH]ppA (lane 8). Similarly, when the reaction was allowed to proceed for 2 min in the presence of ATP after a first step in the presence of p[NH]ppA, the pattern observed (lane 7) was very similar to that in the analogous experiment without ATP and p[NH]ppA in the first step (lane 5). These observations clearly implied that the initiation events observed in the presence of p[NH]ppA were due to the endogenous ATP.

In the two-step assays of Fig. 7, in which ATP was present in the first incubation, the band corresponding to the H-strand runoff was not visible after a 2-min incubation without or with p[NH]ppA (Fig. 7a, lanes 3 and 6), and it was barely visible after a 4-min or 10-min second incubation (not shown), even after long exposures of the autoradiogram. By comparison with the patterns of Fig. 2, it appears that the labeling of the H-strand runoff was very inefficient or delayed relative to that of the L-strand runoff.

DISCUSSION

Previous work with a partially purified RNA polymerase had shown that the optimum ATP concentration for human mtDNA transcription was 0.5–1.0 mM, with a sharp drop of transcriptive activity at 2.5 mM (4). In the present investigation, the ATP concentration allowing maximum efficiency of transcription of both H-strand and L-strand mtDNA in a

crude mitochondrial lysate was found to be much higher, around 2.5 mM, with a shoulder around 0.5–1.0 mM in the ATP response curve. This higher ATP requirement of mtDNA transcription in a mitochondrial lysate did not appear to be due to nonspecific ATP degradation resulting from the ATPase(s) and phosphatase(s) activities expected to be present in this crude fraction.

A plausible, although not exclusive, interpretation of the broad range of ATP concentrations allowing mtDNA transcription in a mitochondrial lysate, and particularly of the high transcriptive activity occurring at >1 mM ATP levels, is the heterogeneity of the RNA synthesizing units operating in the lysate. The difference in the ATP response curves for H-strand and L-strand transcription most probably reflects some differences in the components that make up the enzymatic machineries involved (see below). However, it is possible that differences in the promoter sequences (6) also play a role.

The main conclusion of this work is the identification of a specific requirement for ATP, which cannot be satisfied by p[NH]ppA, at an early step of the transcription process. Evidence for this specific requirement has come from two observations. In the first place, p[NH]ppA, although by itself unable to support transcription of either H-strand or L-strand, greatly stimulated mtDNA transcription at both promoters in the presence of a low concentration of ATP. This result implied that p[NH]ppA failed to support either an early event in transcription (formation of preinitiation complex or initiation) or elongation, but not both. Second, evidence provided by kinetic experiments involving two-step reactions clearly indicated that either the formation of the preinitiation complex or that of the first few phosphodiester linkages was not supported by p[NH]ppA; in fact, the short G-arrested transcripts formed in the presence of the analog were as abundant as those observed in its absence and could be accounted for by the use of endogenous ATP. In view of this result, the observation of enhanced transcription in the presence of ATP and the analog implied that p[NH]ppA could substitute effectively for ATP in the elongation steps. The inability of p[NH]ppA to replace ATP in the initiation of mtDNA transcription *in vitro* could be due to the fact that hydrolysis of the β - γ bond of ATP is required for some step in the transcription initiation process, either as energy source or for the purpose of phosphorylating some specific component of the transcription machinery. While it is clear from the evidence presented in this paper that the specific role of ATP detected here is required for initiation of L-strand transcription, one cannot say conclusively whether this specific role also applies to initiation of H-strand transcription, in view of the low efficiency or delay observed in the latter transcription in the two-step experiments.

The present work has also revealed a different dependence of H-strand and L-strand transcription on the amount of template or mitochondrial lysate used. Since both the L-strand and the H-strand promoter were present in the mtDNA template used in these experiments, the different dependence of the two transcription events on the amount of lysate may indicate the existence of some component of the transcription machinery required for both transcription events, but which has a higher affinity for the L-strand promoter; this component could be present in limiting amount in the lysate, either intrinsically or as a result of loss or inactivation. A competition between the two promoters for

some common component has been reported (6). Alternatively, the different dependence on lysate concentration may indicate the existence in the lysate of distinct components required for the two transcription events, that (those) for the H-strand being in lower concentration. Indeed, the existence of some limiting H-strand-specific component is strongly suggested by the different response of H-strand vs. L-strand transcription to increasing DNA concentrations for a given lysate concentration. The inhibition of L-strand transcription observed at high lysate concentrations may be a nonspecific phenomenon resulting from the presence in the lysate of nucleases, DNA-binding proteins, or competing enzyme activities. The apparent absence of a similar inhibition of H-strand transcription could conceivably result from the compensating effect of increasing amounts of a limiting H-strand-specific component(s).

In conclusion, while the general similarity in the ATP response curves has indicated that the transcription machineries for L-strand and H-strand have common components, in agreement with recently reported observations (18), the different response to DNA concentration of the two transcription events clearly points to the occurrence of H-strand- and, possibly, L-strand-specific components.

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